CONTRIBUTIONS TO THE CYTOLOGY AND PHYLOGENY OF THE SIPHONACEOUS ALGAE.

I. The Cytology of the Gametangia of Codium tomentosum (Stack.).

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(Forty-two Text-figures.)

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Introduction.

The Siphonales are usually described as consisting of those Algae in which the plant body is a coenocyte or a mass of coenocytic branches. Cell walls or septa rarely appear except in connection with the reproductive organs. The plant body may assume various forms, from simple filaments, to some of the most complex forms found amongst the Algae.

Hitherto, algologists have devoted their attention to systematic work on these algae, very few records appearing of cytological investigation. Davis (1904) described oogenesis in *Vaucheria*, and in the same year Ethel S. Gepp described the sporangia of *Halimeda*. Stephens (1899) and Davis (1900, 1903, 1904) discuss the Siphonaceous Algae as a probable ancestral stock from which the Phycomycetous Fungi have been derived. Before such an hypothesis can be definitely accepted, it is necessary that further cytological details amongst the Siphonales themselves should be investigated. Further, a study of the morphology and life cycle of the chromosomes, in connection with the theoretical problem of alternation of generations, is becoming of vast importance at the present day. Yamanouchi, in 1909, drew attention to this vast field of research and the problems with which it abounds.

The classification of the Siphonaceous Algae which seems to be generally accepted at the present day is that of Oltmann. He recognizes two classes.

1. *Siphonocladiales* in which the structure is not wholly coenocytic, division walls appearing at frequent intervals along the filament. In this order are included the Cladophoraceae, Siphonocladiaceae, Valoniaceae, Dasycladiaceae and the Sphaeropleaceae.

2. *Siphonales* in which the plant body is a true coenocyte. This order includes the Codiaceae, Bryopsidaceae, Caulerpaceae and Vaucheriaceae.

This classification recognizes the Codiaceae as the most primitive member of the Siphonales. The reproduction here is the simplest found in the order and takes place either by biciliated zoospores produced in spherical branches, or by fusion of biciliated heterogametes, although zoospores and gametes are known in but few genera. The evolution of a more and more complex plant body is characteristic of the Codiaceae. The Bryopsidaceae are little more advanced. Here asexual reproduction is unknown; sexual reproduction takes place by means of biciliated heterogametes produced in lateral branches.

The Caulerpaceae produce a very complex plant body, the entire plant being coenocytic. The reproduction is so far unknown.

The Vaucheriaceae are recognized as the climax family of the Siphonales. They are freshwater forms. The asexual reproduction takes place by means of a large compound multiciliated zoospore. Sexual reproduction takes place by the union of minute biciliated spermatozoids with a non-motile egg.

The genus *Codium* was first established by Stackhouse in 1795, although the exact nature of the reproductive organs was not described till many years later. Thuret (1850) described the reproductive organs to be of the nature of zoospores produced in sporangia. Later, Berthold described two kinds of zoospores, and maintained that fusion of these was necessary before the young thallus could be formed. They were produced in the same kind of sporangia on different plants, both were of the same general form, only one was larger than the other and of a dark green colour. These he termed the macrozoospores. The others, the microzoospores, were smaller and of a yellowish colour. Since fusion occurred before germination could take place, Berthold established that the macrozoospores were the female gametes and the microzoospores the male. Went (1889) maintained that the plants of *Codium tomentosum* were dioecious, and further, he described the experiments which, he considered, proved conclusively that the macrozoospores germinated parthenogenetically without any fusion with the microzoospores. It is generally agreed that this statement requires further confirmation. Oltmann (1904) observed and figured the fusion of gametes and the subsequent development of the zygote which germinates immediately.

Codium tomentosum is a cosmopolitan species. It consists of an elongated cylindrical, spongy thallus, branching dichotomously and fixed to the substratum by means of rhizoids. The thallus consists of a densely interwoven mass of medullary filaments which give rise to numerous club-shaped palisade branches. The filaments are composed of a parietal layer of protoplasm which lines the mucilaginous cell wall and surrounds a large vacuole. In this parietal layer of protoplasm, numerous small nuclei and chromatophores are scattered. The latter are irregularly distributed through the protoplasm; usually at the base of the branches they are few, while at the apices they are usually so closely aggregated as to give a green colour of an intensity almost equal to black. This is so much so that it completely obliterates the colourless hyaline protoplasm. Usually a good deal of starch is present, associated with the chromatophores. Division walls are absent from the filaments and normally only arise in connection with the reproductive organs. It has been claimed that the coenocytic structure has been gradually attained by the formation of fewer and fewer partitions in a succession of filamentous plants.

The palisade branches are usually several times the diameter of the medullary branches which produce them. The palisade branches in their turn produce the gametangia, sometimes borne singly, but often produced in a row on either side of the palisade branch and are cut off from the latter by a plug-like wall (Text-fig. 1). As the gametangia mature, they drop off and a scar of attachment is left on the palisade branch. The writer's observations confirmed the statement that male and female gametangia are produced on different plants, i.e. the plants are monoecious.

The material for the present investigation was collected at Bondi, N.S.W. Here it grows profusely just outside the limit reached by the low tide. The material was fixed in the field in a weak chromo-acetic solution: Chromic acid

0.4 g., Glacial acetic acid 1 c.c., Sea water 400 c.c. As the plant is of such a spongy nature, an air pump was used to remove as much of the air as possible to facilitate the infiltration of the fluid. The fixative was allowed to act for about 24 hours. Material was then washed thoroughly in sea water and passed in the usual method into paraffin. Sections were made varying from 3μ to 5μ in thickness and stained, Flemming's triple stain and Haidenhain's Iron-alum Haematoxylin being employed. The latter staining process gave the better results.

Investigation.

The gametangia are of two kinds, male and female, and are produced on different plants. The male gametangia are very much smaller than the female. Both contain very dense, coarse, granular cytoplasm, in which are embedded numerous nuclei and chromatophores. This is enclosed by a wall, distinctly two-layered, the outer layer being firm and thin, the inner thick and capable of a great degree of swelling.

The gametangia are produced from the palisade branch in the following manner. A small protuberance is formed from the wall of the branch and into this a certain amount of protoplasm together with nuclei and chromatophores flows. The protuberance enlarges and the protoplasmic flow continues until a certain limit is reached, when the gametangium is cut off from the palisade branch by a thick septum. Definite evidence of this flow is recorded in the gametangium itself by the arrangement of the chromatophores, all of which have their long axis arranged almost parallel to the long axis of the gametangium, giving it a striated appearance. The nuclei, also, become elongated with their long axis arranged in the same direction. The protoplasm is evenly distributed, no vacuoles being visible from the beginning of growth. When the cross partition is formed, the gametangium is filled with colourless granular cytoplasm, nuclei, and very numerous chromatophores (Text-fig. 2).

When development has proceeded thus far, certain changes occur in the game-tangia which are essentially similar in both male and female.

The gametangium at first contains numerous very small, elongated nuclei, each containing a nucleolus, a very fine linin reticulum and surrounded by a thin nuclear membrane. The nuclei once more assume a rounded form. Very soon certain nuclei commence to disintegrate; the reticulum and membrane disappear, leaving the nucleolus which stains very brightly, but finally it also disintegrates.

Co-ordinate with this nuclear degeneration, is the enlargement of certain other nuclei which are destined to undergo a heterotypic division.

A. *Mitosis in Female Gametangium.*

The nuclei as they enter the gametangium are small and elongated in the direction of flow of the protoplasm. In this condition they are more numerous at the apex of the gametangium than at its base. As soon as the dividing septum is formed, however, they lose their elongated appearance and become rounded once more, at the same time becoming more evenly distributed throughout the protoplasm. So far as the writer was able to observe, no definite number of nuclei enters the gametangium. The nuclei at this period have a thin nuclear membrane, a fine chromatin net work and a small nucleolus (Text-fig. 3). Some of the nuclei now commence to disintegrate, the nucleolus persisting till the last and then finally disappearing (Text-fig. 4). These supernumerary nuclei

have completely disappeared before the mitoses which take place in the game-tangium are completed. Certain other nuclei enlarge considerably; at the same time the chromatin thread becomes very much more distinct. The nucleolus does not increase in size (Text-fig. 5). The selection of functional nuclei seems to be related to their orientation with regard to certain dynamic centres present within the gametangium. It is the nuclei in the vicinity of these centres which survive, and those further away perish. The nature of these bodies is indicated in Text-fig. 8. They are not very large, but absorb the stain with avidity so that they are easily recognizable in preparations. These bodies appear to be of the nature of dense protein centres surrounded by clear, colourless zones. Several such bodies are present in each gametangium. Observations indicated that they nourish several nuclei in their vicinity. They seem to be of a chemotactic and nutritive nature. A peculiar feature is that they are small bodies, smaller than the nuclei themselves. The bodies present to the writer very much the same structure and serve the same function as the coenocentra described by Davis (1903) in *Saprolegnia* and by Stevens (1899) in *Albugo*. The term coenocentrum, in these fungi, is applied to the centre of a coenocytic body, such a centre being connected with definite processes in oogenesis. The coenocentrum, also, is a very large structure, very much larger than the nuclei. This is precisely the reverse of the writer's observations in *Codium*. Hence, it would not seem justifiable to term these bodies, present in *Codium*, coenocentra.

As the nuclei in the vicinity of these bodies enlarge they are seen to contain a thin, ragged and much branched chromatin thread. There is no manifestation of polarity of the nucleus. Soon it becomes evident that the network is composed of very small chromatin knots connected together by irregular fibrils (Text-fig. 5). The chromatin knots become very much larger, especially those near the nuclear membrane. Co-ordinate with this, the nuclei increase in size still further; ultimately the chromatin thread is located at one side of the nucleus. This growth period of the nucleus eventually results in its becoming two or three times its original size, but as the chromatin mass does not increase at the time, it becomes left at one side, giving the typical appearance of the so-called synapsis, as indicated in Text-fig. 6. This stage forms a striking feature of the cytology of the reduction division, but appears nowhere else in the whole life history of the plant. Owing to the minute size of the nuclei it is difficult to determine the factors responsible for synapsis, but certain it is that associated with it is a considerable increase in the size of the nucleus. At the same time, the threads of the chromatin undergo considerable modification, they become very much thicker, and the general appearance seems to indicate a shortening and thickening of the threads. The chromatin thread thus becomes more sharply defined, but still has the appearance of being irregularly interwoven even in synapsis. At this stage the nucleolus may be recognized associated with the chromatin threads. Soon the chromatin threads commence to loosen, and certain of them project into the nuclear cavity (Text-fig. 7). At the same time the chromatin threads have continued their processes of shortening and thickening. The exact mechanism of the separation of the individual chromosomes could not be clearly determined. Whatever the method employed, ten pairs of bivalent chromosomes are to be observed in the nucleus in early prophase (Text-fig. 9). These are usually distributed through the nuclear cavity.

In late prophase, the nucleolus is sometimes to be observed in the nucleus in a fragmentary condition. The spindle fibres begin to make their appearance,

and attach themselves to the chromosomes, thus the achromatic spindle, with its equatorial plate, is established. The spindle is long and narrow, and its long axis is invariably parallel to that of the gametangium (Text-figs. 10, 12). A distinct membrane surrounds the spindle at this period, but on account of the minute size of the nuclei, it is difficult to determine whether this is the original nuclear membrane. If the membrane is of this nature, it would follow that the spindle would be intranuclear, but that is by no means certain. Centrosomes with radiations were not observed. Davis (1903, p. 230) notes the absence of these structures from the intranuclear spindles of *Saprolegnia*. Centrosomes may be present in *Codium tomentosum*, but the granular nature of the cytoplasm, the large chromatophores, and the avidity with which they stain, would make such minute structures difficult to determine.

The chromosomes are long rod-shaped structures, hence their arrangement on the equatorial plate is not always clear. In polar view of the various stages their number may be fairly accurately determined. The result of making a count of the number of chromosomes in the polar view of the metaphase of several hundred median sections proved them to be ten in number, but these are bivalent (Text-fig. 11). In metaphase the two halves of the bivalent chromosomes separate and pass towards the poles. Anaphase follows; the chromosomes begin to straighten out and are arranged near the poles (Text-fig. 13). After telophase, the two daughter nuclei are organized, the chromosomes lose their individuality

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Text-fig. 1.—A palisade branch bearing a gametangium and showing the scars of attachment of older gametangia (× 60).

Text-fig. 2.—Young gametangium (female) indicating the distribution of nuclei and chromatophores (× 150).

Text-figs. 3-21.—Mitosis in Female Gametangium.

3-14.   First Division.

3.—This indicates the condition of the nucleus at the period of its entrance into the gametangium (× 2000). 4.—A degenerating nucleus (× 2000). 5.—A functional nucleus indicating increase in size and the nature of the chromatin reticulum which consists of granules connected together by delicate fibrils (× 2000). 6.—Nucleus in synapsis (× 2000). 7.—This indicates the loosening of the chromatin mass in synapsis, the chromatin threads projecting into the nuclear cavity in the form of loops (× 2000). 8.—This indicates the nature of the bodies which appear to select the functional nuclei. In the vicinity are three nuclei in synapsis (× 2000). 9.—Prophase: 20 chromosomes are developed and are distributed throughout the nuclear cavity, and are grouped in pairs (× 2000). 10.—Metaphase: the spindle has developed and the chromosomes are arranged on the equatorial plate (× 2000). 11.—Polar view of nucleus in metaphase indicating the presence of ten chromosomes (× 2000). 12.—A gametangium indicating the simultaneous division of the nuclei which are in metaphase. The long axes of the spindles are parallel to that of the oogonium (× 150). 13.—Anaphase: the two sets of daughter chromosomes are passing towards the poles (× 2000). 14.—The two daughter nuclei are organized and the chromosomes are losing their identity. The spindle fibres are still faintly visible connecting the two nuclei (× 2000).

15-21.   Second Division.

15.—Late prophase: a number of chromosomes have emerged from the ragged reticulum. The nucleolus is still present (× 2000). 16.—Metaphase: the two sets of daughter chromosomes are proceeding to the poles (× 2000). 17.—Polar view of prophase, indicating that there are 10 chromosomes associated with this division (× 2000). 18.—Anaphase: the two sets of daughter chromosomes are proceeding to the poles (× 2000). 19.—Longitudinal section of a mature gametangium indicating the nature of the female gamete primordia (× 270). 20.—The female gamete has a rounded contour. Nucleus is in a central position (× 610). 21.—The nucleus of the female gamete has taken up a peripheral position (× 610).
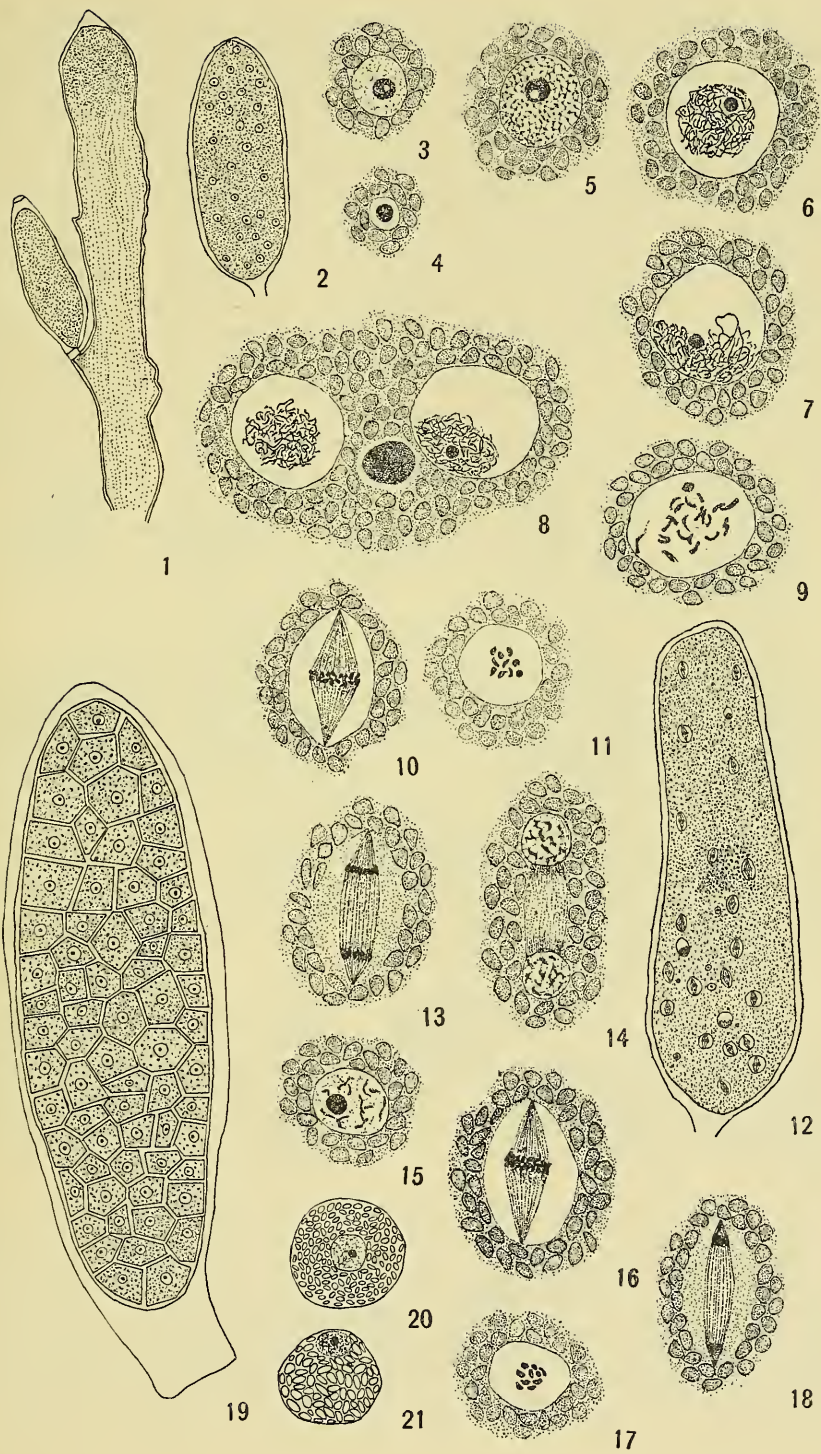
and the ragged reticulum is once more established. The daughter nuclei are much smaller than the parent nucleus from which they were formed (Text-fig. 14).

After a short period of rest the daughter nuclei undergo another division, which is essentially similar, except in the number of chromosomes, to the ordinary mitotic divisions throughout the plant. In early prophase, ten univalent chromosomes are differentiated out from the ragged reticulum (Text-fig. 15). In late prophase the achromatic spindle, with its equatorial plate, is established. A thin membrane surrounds the spindle, but soon undergoes rapid degeneration. No centrosomes or radiations were observed associated with the spindle. The long axis of the spindle of the second division is also parallel to that of the gametangium. In metaphase the chromosomes divide and pass to the poles (Text-fig. 16). Where the splitting of the chromosomes occurs which provides for the second division could not be accurately determined. Anaphase (Text-fig. 18) and telophase follow, and the daughter nuclei are organized, each with its well-defined reticulum and nuclear membrane. In prophase, ten chromosomes are differentiated out in polar view, and this number remains constant throughout the mitosis (Text-fig. 17). The daughter nuclei are very small compared with the parent nucleus of the first division.

These are the only mitoses which occur in the organization of the female gametangium. They are simultaneous in all the nuclei concerned.

Text-figs. 22-35.—Mitosis in the Male Gametangium.
22-29. First Division.
22.—A functional nucleus indicating the well defined chromatin reticulum which is differentiated into granules connected together by delicate fibrils. A degenerating nucleus is also indicated here (× 2000). 23.—Nucleus in synapsis (× 2000). 24.—This indicates the loosening of the chromatin mass in synapsis; certain of the chromatin threads are seen projecting into the nuclear cavity in the form of loops (× 2000). 25.—Prophase: the chromosomes are approximately 20 in number and are grouped together in pairs (× 2000). 26.—a. Metaphase: chromosomes are just separating to pass towards the poles (× 2000). b. Metaphase: in the vicinity of the spindle is one of the structures which select the functional nuclei (× 2000). 27.—Longitudinal section of the gametangium indicating the simultaneous division of nuclei which are in this instance in metaphase (× 150). 28.—Polar view of chromosomes at metaphase indicating that they are ten in number (× 2000). 29.—Late anaphase: the two sets of daughter chromosomes are nearing the poles of the spindle (× 2000).

30-35. Second Division.
30.—The nucleus at the beginning of division. The reticulum is being differentiated into numerous chromatin knots (× 2000). 31.—Metaphase: the chromosomes have separated (× 2000). 32.—Polar view of late prophase indicating that ten chromosomes are present (× 2000). 33.—Longitudinal section of the male gametangium indicating the nature of the male gamete primordia (× 270). 34.—The male gamete before liberation; it has a rounded contour and the nucleus is in a central position (× 610). 35.—The nucleus of the male gamete has migrated and taken up a peripheral position (× 610).

Text-figs. 36-42.—Mitosis in Vegetative Coenocyte.
36.—A small nucleus of the central coenocytic threads of the plant indicating scarcity of chromatin material (× 2000). 37.—A nucleus of a palisade branch preparing for division; the chromatin material is more distinct, chromatin knots are being organized (× 2000). 38.—This indicates the nature of the spireme which is beginning to segment (× 2000). 39.—Late prophase (polar view); chromosomes are differentiated and are 20 in number (× 2000). 40.—Metaphase: the chromosomes are arranged on the equatorial plate (× 2000). 41.—Same as the above (Text-fig. 40) viewed from the poles, indicating that 20 chromosomes are present (× 2000). 42.—Telophase: the two sets of daughter chromosomes have reached the poles and are losing their identity (× 2000).

As soon as the daughter nuclei are formed, they separate and become evenly distributed throughout the cytoplasm of the gametangium. The protoplasm surrounding each nucleus, taking the latter as a centre of formation, becomes marked out by cleavage planes. Cell membranes make their appearance for the first time within the gametangium; each nucleus with its attendant cytoplasm is a female gamete primordium (Text-fig. 19), and becomes transformed into a single female gamete. At first, these are hexagonal in outline and fit closely to one another, but later, when ready for liberation, they separate and become rounded (Text-fig. 20). The nucleus then migrates from the centre of the gamete to a peripheral position (Text-fig. 21) and the gamete becomes pear-shaped. Cilia appear at the pointed end of the gamete in proximity to the nucleus. The gametangium opens by a pore at its apex through which the gametes escape. The liberation of the gametes is due to the shrinking of the wall of the gametangium, brought about by exposure and relaxation of pressure

at low tide. There are several hundred gametes produced in each gametangium.
As there are countless numbers of gametangia produced in each branch of the
plant, it follows that the gamete production is enormous.

B. *Mitosis in the Male Gametangium.*

The male gametangium is very much smaller than the female, but the pro-
cesses connected with the organization of the gamete are similar in both. When
the septum is formed, separating the male gametangium from the palisade
branch which bears it, certain of the nuclei degenerate, while others enlarge
considerably. There is an apparent marked increase in the amount of chromatin
in the surviving nucleus, but no increase in the size of the nucleolus (Text-fig. 22).
The nuclei which survive are those in the vicinity of certain bodies of the same
nature as those described as occurring in the female gametangium. Such a
structure is indicated lying close to the spindle in Text-fig. 26, *b*. Co-ordinate with
the increase in size of the surviving nuclei, small granules make their appear-
ance on the ragged reticulum, these being connected by delicate fibrils. With
further increase in the size of the nucleus, the chromatin threads become
eccentrically placed in the nuclear cavity, presenting the appearance of typical
synapsis (Text-fig. 23). The nuclei are too minute to observe the detail of forma-
tion or structure in synapsis. The chromatin threads undergo shortening and
thickening so that they are very much more distinct in synapsis than in the
pre-synaptic stages. The chromatin in synapsis presents the appearance of a mass
of closely interwoven threads (Text-fig. 23). Later the chromatin threads
commence to loosen, and certain of them may project into the nuclear cavity
(Text-fig. 24). The chromatin thread arranged in synapsis must undergo further
shortening and thickening, since the chromosomes when they are formed are
short, thick structures. In prophase, 20 chromosomes were counted; these were
grouped in pairs, each representing a pair of bivalent chromosomes (Text-fig. 25).
These chromosomes become distributed in the region of the centre of the nucleus.
In late prophase the spindle fibres appear; they attach themselves to the
chromosomes, and the achromatic spindle, with its equatorial plate, is established.
The spindle is long and narrow; it is surrounded by a thin membrane, well
defined in metaphase, but during anaphase it breaks down, disintegration com-
mencing at the poles. No centrosomes or radiations were observed in connection
with the formation of the spindle. The long axis of the spindle is parallel to
that of the gametangium (Text-figs. 26, 27). In metaphase the chromosomes
separate and pass towards the poles; ten chromosomes were counted in polar
view of this stage (Text-fig. 28). Anaphase follows (Text-fig. 29); at telophase, the
two sets of daughter chromosomes are crowded closely together and nuclear
membranes are formed. The chromosomes lose their identity, and a ragged
reticulum is formed; the nucleoli also reappear.

After a short period of rest, the daughter nuclei undergo another simultaneous
division, similar to that which occurs in the female gametangium. There are
ten chromosomes associated with the various stages of this division, as indicated
in Text-figure 32, which represents a polar view of late prophase; with this
reduced number the nucleus passes into telophase. The daughter nuclei are
very small. The nature of this division is indicated in Text-figures 30-32.

When this mitosis is complete, cleavage planes are formed in the cytoplasm,
cell membranes make their appearance surrounding each nucleus and its attendant
cytoplasm. This is the first appearance of cell walls within the gametangium.

The nucleus with its attendant cytoplasm becomes a male gamete primordium, each giving rise to a single male gamete. These are at first hexagonal in outline and lie close together (Text-fig. 33), but later they separate and become rounded in outline (Text-fig. 34). Co-ordinate with these changes in the shape of the gamete, the nucleus which is originally centrally placed, moves, and takes up a peripheral position (Text-fig. 35). Cilia are developed on the side of the gamete near the nucleus. Liberation of the gamete is effected by the same means as has been already described in the female gametangium. The male gametes are very much smaller than the female and of an orange colour. Countless hundreds of gametes are produced in each gametangium.

C. *Division of the Nuclei of Vegetative Coenocyte.*

The nuclei of the coenocytic threads of the central region of the thallus are very small, in fact they are little larger than the chromatophores themselves (Text-fig. 36). They contain a small nucleolus and a scarcely visible chromatin reticulum. These nuclei are scarcely satisfactory for a study of nuclear conditions in the coenocyte. The nuclei occurring in the outer branches, especially those of the palisade branches, however, are somewhat larger. A thorough study of a typical mitosis was made in order to make an estimate of the number of chromosomes present in the vegetative body grown under normal conditions. Vegetative mitosis agrees in essentials in both male and female plants.

The nuclei occurring at the apices of the palisade branches contain a small nucleolus and a faint chromatin reticulum. In early prophase the nucleolus commences to degenerate, while the chromatin reticulum becomes very much more prominent and dispersed over the nuclear cavity. Chromatin knots become visible (Text-fig. 37), resulting in the reticulum gradually becoming transformed into a convoluted spireme (Text-fig. 38). The thin spireme thickens and later splits to form chromosomes. These become regular in size and shape (Text-fig. 39).

In late prophase spindle fibres make their appearance and numerous chromosomes are arranged on the equatorial plate. In metaphase the chromosomes split and each half proceeds towards the poles of the spindle (Text-fig. 40). The nuclear membrane is only to be observed in the equatorial region of the nucleus; in anaphase it has completely lost its identity. In late prophase and in polar view of metaphase, 20 chromosomes were recognized as being present (Text-fig. 41). They are often difficult to count on account of their number in comparison with the small size of the nucleus. It seems probable that the splitting of the parent chromosome occurs when they have become arranged on the equatorial plate. In anaphase, the two sets of daughter chromosomes proceed to the poles; telophase follows (Text-fig. 42). When they reach the poles, nuclear membranes are formed, the chromosomes lose their identity and two typical resting nuclei result.

General Considerations.

The present communication is of interest in that it indicates that alternation of generations is present associated with a fairly primitive form of sexual reproduction; the gametes are isogamous and but little removed from the asexual zoospore from which they are supposed to have been derived.

As the history of the discovery and establishment of alternation of generations among the Thallophytes has been so admirably set forth by Yamanouchi (1912), it would be superfluous to relate it here, except to quote a few

of that author's words which briefly summarize the present extent of the investigations in this direction (p. 485): "The existence of an alternation of generations among the Thallophytes, though somewhat obscure in the green algae on account of insufficient investigations, has been clearly proved by cytological study on *Fucus* (Yamanouchi, 1909), *Cutleria* (Yamanouchi, 1912), and *Dictyota* (Williams, 1904) among the brown algae, and in *Nemalion* (Wolfe, 1904), *Polysiphonia* (Yamanouchi, 1906), *Griffithsia* (Lewis, 1909) and *Delesseria* (Svedelius, 1911) among the red algae." In addition to these, Williams established the presence of an alternation of generations in *Laminaria* in 1921.

The investigation recorded in the present communication indicates that alternation of generations occurs in the life history of *Codium tomentosum*, although it is to be regarded as either a cytological alternation or as an aberrant type. The nuclei of the coenocytic threads of the vegetative portions of the plant contain 20 chromosomes. The nuclei of the gametangia, both male and female, undergo two divisions, in the first of which 10 bivalent chromosomes are present; the second division has the same number of univalent chromosomes.

There are two possibilities as to the nature of the alternation of generations in *Codium*. In the first instance, since the nuclei of vegetative coenocytic branches contain the diploid number of chromosomes, they may be regarded as sporophytic in character. The nuclei of the young gametangium (before division occurs) may be compared with the spore mother cells of higher plants (in *Codium*, cell walls do not appear separating the spore mother cells, but they are enclosed by a common membrane). Certain of the nuclei degenerate; the surviving nuclei undergo two divisions. The daughter nuclei which result from the second division may be compared with the spores of higher plants, that is, they are the beginning of the haploid generation. Still enclosed, they germinate to form the gametophytic generation. Each nucleus of this generation, with its attendant cytoplasm, by cell formation becomes a gamete primordium, each eventually becoming a single uninucleate gamete. These gametes are liberated, fuse and germinate; the sporophytic generation is once more produced. This implies that alternation of generation in this type is purely cytological with probably a primitive type of gametophyte. The main objection to this explanation is that it is impossible to determine in the haploid generation where the haploid spore ends and the gamete primordium begins.

Cytological alternation has been recorded in *Fucus* (Yamanouchi, 1909), but here the gametophytic generation, when initiated, undergoes one mitosis in the oogonium and four in the antheridium.

The second possibility is that *Codium* may be regarded as an aberrant type so far as the generally accepted ideas of alternation of generations are concerned. This may be due to the fact that the reproductive organs are primitive in character; the gametes are but little removed from the asexual zoospore from which they are supposed to have been derived. The term "sporophyte" is usually applied to that portion of the life history which contains the diploid number of chromosomes and produces the spores; these in their turn produce the "gametophyte" or the haploid generation which produces the gametes. Morphologically, spores are not produced in the life cycle of *Codium*, hence the suggestion that, since the plant produces haploid gametes directly, but is itself diploid in nature, it may be regarded as a diploid gametophyte.

The results of the investigations of many cytologists in other parts of the plant kingdom have led to the establishment of many definite facts with regard

to the phenomenon of alternation of generations. Taking these into consideration, the best explanation of its occurrence in *Codium tomentosum* seems to be that it is cytological in character, with probably a primitive type of gametophyte.

The various details accompanying gametogenesis in *Codium tomentosum* also present considerations of great interest. All the surviving nuclei in the gametangium, both male and female, are to be found at the same stage of mitosis at precisely the same time, that is, simultaneous division of nuclei occurs. This has been recorded from various parts of the plant kingdom. Stevens (1899) and Davis (1903) emphasize its occurrence in *Albugo* and *Saprolegnia* respectively amongst the Phycomycetous Fungi. Yamanouchi has recorded it in the oogonia and antheridia of *Fucus* (1909), and in oogenesis, spermatogenesis and zoosporogenesis in *Cutleria* (1912). In *Codium* this simultaneous division is of the nature of a reduction division necessary in the organization of the gametes. Nuclei of all ages enter the gametangium, so that this division not only maintains constancy in the number of chromosomes, but also serves to bring the nuclei to the one age once more.

Previous to these mitoses, degeneration of certain nuclei occurs, these nuclei representing supernumerary sperm mother nuclei.

The factors responsible for the selection of the functional nuclei are very interesting. Small granular bodies which stain deeply, and of a different nature from the ordinary cytoplasm, make their appearance, embedded in the cytoplasm. They first appear as visible structures at the period when certain of the nuclei are degenerating and others are preparing for division. It is the nuclei in the vicinity of these bodies which enlarge and undergo a heterotypic division, that is, the surviving nuclei owe their preservation to their orientation with regard to these structures. They not only nourish one nucleus in their vicinity, but several. They are not permanent structures of the gametangium, but are of a transitory nature. They appear associated with the most active periods of gametogenesis and disappear when the first nuclear division is well advanced. They represent centres of dynamic activity, their function resembling that of the coenocentra described by Davis (1903) in *Saprolegnia*, and the single coenocentrum by *Albugo* (Stevens, 1899). Their earlier appearance in *Codium tomentosum* is probably related to the differences in the processes of formation of the structures with which they are associated in these types. A rather remarkable feature in *Codium* is their small size as compared with the nuclei. Whether they are vestigial or primitive structures amongst these algae is impossible to determine.

Much interest has become centred around this region of the Siphonales (*Codium*) of recent years on account of the fact that it is these plants with simple forms of gametangia, producing uninucleate swarming spores, which have been looked to as the possible ancestors of the Phycomycetous Fungi such as the Mucorales, Saprolegniales, and Peronosporales.

It is generally agreed that the coenogamete is homologous with the gametangium. In the light of the present communication, it would seem that a further study of the nucleus of the Phycomycetous Fungi with regard to the significance of the mitoses which occur during oogenesis and spermatogenesis of these types is very desirable. Both Stevens (1901, p. 242) and Davis (1903, p. 251) consider the nuclear divisions of the oogonia and antheridia of *Albugo* and *Saprolegnia* respectively are not of the nature of reduction divisions, but are probably phylogenetic reminiscences, essential for the increase in the number of gametes in ancestral forms. Should this remark be proved to be correct, it follows that

the gametangia of *Codium*, at all events, are not homologous with the coenogametes of these Fungi, since such an homology would demand an agreement even in the details of cytology.

Summary.

1. The filaments which go to make up the plant body of *Codium* are coenocytic in structure; the nuclei of these coenocytic threads contain 20 chromosomes.
2. Gametangia are borne on the palisade branches; the plants are monoecious.
3. Certain nuclei of the gametangium (both male and female) undergo degeneration; others enlarge considerably and undergo two nuclear divisions.
4. The nuclear divisions of the gametangium are simultaneous in all nuclei concerned.
5. Selection of the functional nuclei is closely associated with the presence of a varying number of bodies, of the nature of coenocentra, within the gametangium.
6. The first of the nuclear divisions which takes place within the gametangium is heterotypic in character. Ten chromosomes are present during this division, but these are bivalent.
7. The same number of univalent chromosomes are to be found associated with the second division; hence, the daughter nuclei associated with this division contain the reduced number of chromosomes.
8. The achromatic spindles are in all instances probably intranuclear and without centrosomes or radiations.
9. The period during which ten chromosomes are present may be regarded as the haploid stage; gametophyte generation is probably a primitive type.
10. Alternation of generations in *Codium tomentosum* is purely cytological in character.

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